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STUDIES ON THE PERITONEAL ABSORPTION OF PARTICULATE MATTER

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CONTENTS

I. HISTOLOGICAL OBSERVATION OF THE PERITONEUM PARTICIPATING THE ABSORPTION

Absorption through the peritoneum is of considerable interest clinically in understanding the resolution of effusion, the spread of infection and the metastasis of malignant tumors occurring in the peritoneal cavity.

Since v.Recklinghausen (1863) discovered that injected particles into physiological peritoneal cavity of a rabbit were rapidly absorbed through the diaphragm, numerous studies about this have been made by Klein, Burdon, Sanderson (1872), Muscatello (1895), Glober (1901), Küttner (1903), MacCallum (1903), Buxton (1906), Walter (1921), Bolton (1921), Magnus (1923), Higgins, Bain (1930), Higgins, Beaver, Lemon (1931), Allen, Vogt (1937) and Simer (1948).

However, these literatures were unsatisfactory from the morphological point of view.

Under Prof. Dr. Kihara, many investigators have systematically studied this problem and consequently discovered following facts that particles injected into the peritoneal cavity penetrated the intercellular cement substances of the diaphragmatic peritoneal endothelium and moved through the sieve-like constitution to the endothelium of the lymphatic vessels. This sieve-like constitution, which was formed by both collagen and reticulum fibers in the subendothelial connective tissues of the diaphragm, was named macula cribriformis by Prof. Dr. Kihara.

Thereafter, this structure was discovered on the parietal pleura, pericardium, mediastinal pleura and omentum, in which absorption of the particles into lymphatics was observed. In other words, macula cribriformis formed prae-lymphvascular fluid path in the subendothelial connecsive tissues.

Whereas, Matsuda (1951) postulated that India ink injected into the pleuroperitoneal cavity of some kinds of vertebrates was absorbed in various sites of

serous surface as follows; membrana subvertebralis, mesenterium and serosa of intestine in a wels; serosa of stomach in newts; membrana subvertebralis, mesenterium and serosa of large bowel in toads; mesenterium and serosa of the intestine in a lizard and a tortoise; serosa of the stomach and the diaphragm in fowl; mainly the diaphragm and the omentum, slightly mesenterium and mesoovalium in rabbits. The subendothelial structure of these absorption sites is mesh-work composed of both collagen and reticulum fibers.

This study was undertaken to investigate whether macula cribriformis could be detectable in the peritoneal cavity of mammalia besides diaphragm and omentum by accepted method.

METHOD AND MATERIAL

1) Animals. Albino rabbits weighing approximately 1.5–2.0 kg were used.

2) Technical procedures. India ink, (finely divided carbon particles disolved in 5% glucose solution) was injected into peritoneal cavity through a fine vinyl tube inserting upper abdominal wall. Injected volume of the dye was 25 cc per kg of bodyweight.

Animals were bled to death at 15 minutes to 48 hours after injection of India ink. Routine gross and microscopic studies were performed.

For the purpose of morphological study of the parietal peritoneum, diaphragm, omentum and mesenterium, care was taken to peel them out from the underlying tissues as thinly as possible and they were stretched out over a clean glass slide. After being dried in the room temperature, these materials were fixed by 10% neutral formalin and usually stained with Bielschowsky-Maresch silver method, occasionally they were stained with hematoxylin and eosin or May-Giemsa stain.

Liver, Lgll. sternalis cranialis, mediae and caudalis, lymph nodes in the peritoneal cavity - - especially, Lgll. cardiacae, Lgll. art. pancreaticolienalis, Lgll. art. hepaticae, Lgll. mesentericae craniales, Lgll. a. mesentericae caudalis, - - Lgll.iliacae, Lgll. renalis, Lgll. poplitae and Lgll.axillares were examined in all animals as control materials for the Part II and III. They were embeded in paraffin after fixation and sections were stained with hematoxylin and eosin.

The lymph nodes in thorax - - Lgll. oesophagicae, Lgll. intercostales dorsales, Lgll. mediastinales v. cavae craniales dextrae, Lgll. aortae and Lgll. aortae thoracales caudales lat. - - were examined only macroscopically.

EXPERIMENTAL RESULT

After washing out India ink in the abdominal cavity of a sacrificed rabbit, definite necropsy examination was performed.

The features of peritoneal absorption were as follows.

1) Diaphragm (Fig. 1, 2); Absorption from peritoneal diaphragmatic surface was conspicuous in all animals. In the pars tendinea of diaphragm. India ink formed radial black stripes between tendon bundles, which extended in pars muscularis, becoming obscure gradually.

The cell boundaries of the diaphragmatic peritoneum were outlined here and there by carbon particles. Network of subendothelial lymphatic vessels containing India ink was visible except two animals which had the longer intervals from injection to death. After entering into subdiaphragmatic lymphatics, the particles reached the lymphatic-venous connection mainly through the lymphatic trunks running up behind the sternum and partly through the lymphatic vessels running up in the mediastinum.

And the dorsal mediastinum had not the lymphatics containing the particles. Silver stained specimen revealed that macula cribriformis, which was mesh of reticulum fibrils with collagen frame, was distributed in both pars tendinea and pars muscularis of the diaphragm. The former was arranged regularly in parallel with tendon bundles and the latter was distributed irregularly between muscle bundles forming like beehive. The macula cribriformis were less in number and deformed with sparse reticulum fibrils in the marginal area of the diaphragm. Divided carbon particles of India ink were adherent to fine reticulum fibrils of the macula cribriformis, through which the particles were gathered in the subendothelial lymphatics.

2) Omentum (Fig. 3) : Though omentum was greyish coloured at 15 minutes after injection, it was found microscopically that absorption from omental surface was very vigorous at 2 to 4 hours after injection. In these cases, carbon particles were mostly accumulated in the milky spots, where the particles surrounded the venules and emerged in the lymphatics. After 24 hours or more, free carbon particles on the membrane decreased and macrophages ingulfing them were eminent.

The omentum was composed of wavy collagen fibers, which were woven each other, and straight elastic fibers. In the meshes of collagen fibers, fine fibrils were proved, but they did not form a true net work as macula cribriformis, nor revealed the argyrophilic nature.

In the milky spots, wavy collagen fibers and fine fibrils mentioned above were woven densely each other with abundant distribution of blood and lymphatic vessels, and cellular elements were numerous. Carbon particles were scattered between these fibers, but were not exclusively adherent to the fine fibrils.

3) Mesenterium (Fig. 4, 5, 6.) : The mesenterium of the small intestine was not coloured with India ink within 1 hour. After two hours or more, some specks, lightly coloured greyish, appeared along the mesenteric vessels near the attachment of the intestine. These specks could not be rubbed out with gauze. And there was no speck in the thinner part aloof from the vessels. After 24 to 48 hours, these specks were more deeply coloured by the accumulating phagocytes which ingulfed the particles. Microscopically, the carbon particles accumulated in these specks were adherent to the mesenteric surface, part of which entered into the subendothelial tissue. Lymphatic absorption from these sites could be proved. A few venules were surrounded by the particles, but not containing them.

The mesenterium of the large intestine, especially sigmoid, had greyish coloured specks in some instances more evidently and rapidly than the small intestine. In

them, microscopic observation was similar to that of the small intestine.

Silver stained specimen revealed that the mesenterium was composed of the wavy collagen fibers which were woven each other, more densely near the mesenteric vessels. In the meshes of the collagen fibers were found fibers like down, not so argyrophlic as the reticulum fibrils, along which carbon particles were not accumulated.

A pair of diverticulum of peritoneum by the rectum were always filled up with India ink, but no lymphatic vessel was coloured in adjacent region.

4) The serosa of digestive organ (Fig. 7, 8, 9): The narrowing rings of the caecum in three cases, which were sacrificed 15 minutes, 1 hour and 2.5 hours each after injection, were slightly coloured with India ink. Some of the particles were proved in the subserous tissue, where the particles were arranged near the peculiar type of cells, seeming mesothelial.

Although efficient absorption from these sites via blood stream or lymphatics could not be detectable, this fact seemed very interesting from phylogenetic point of view, as mentioned later.

By the silver impregnation method, the subserous tissue of this region was consisted of wavy collagen fibers which were not woven each other and not ramified with reticulum fibrils.

5) The ventral peritoneum (Fig. 10) was not coloured with India ink in any animal and neither blood nor lymphatic vessels containing carbon particles were visible. Histologically, this part of peritoneum was composed of broad collagen fibers which were stained yellowish brown by Bielschowsky's method and were parallel with M. transversus abdominis. The reticulum fibrils were not found anywhere.

6) In the retroperitoneum (Fig. 11, 12, 13, 14, 15), most of the cases had greyish coloured specks on the fat tissues near the caudal pole of the kidney. The stretched specimen of this coloured parts, staining with hematoxylin and eosin, revealed that injected carbon particles gathered in the interstitium of fat cells and difined the contour of these cells. But blood and lymphatic vessels containing the particles were not seen.

By the silver impregnation method, it becomes evident that the carbon particles seen among fat cells were constantly found at the sites of reticulum fibrils, and that subendothelial tissue of the retroperitoneum, except fat tissue, was composed of wavy collagen fibers with various thickness, which were similar to mesenterium or omentum and quite different from ventral peritoneum. Macula cribriformis was not found anywhere of the retroperitoneum, although fine fibers barely argyrophilic, were seen as well as mesenterium.

DISCUSSION

It is beyond dispute to conclude that particulate matter injected into peritoneal cavity was absorbed exclusively from peritoneal diaphragmatic aspect into lymphatic path.

Absorption through the rest of the parietal peritoneum and the mesenteric folds does not appear to be of much significance quantitatively.

The extent of lymphatic absorption through the omentum, however, has for a long time been debatable. Some investigators have been doubtful against the existence of omental lymphatics. However, TEI (1937), SIMER and CASPARIS (1948) have demonstrated clearly a fairly rich distribution of lymphatics, generally associated with the blood vessels, in the omentum of cats, dogs, rabbits and men.

On the other hand, TAGUCHI (1943) maintained particulate matter was also absorbed into the blood vessels on the omentum. In our experiments, absorption from omental surface was very conspicuous in about 2 hours after injection, when the carbon particles accumulating chiefly in the milky spots were arranged along the wall of venules or filled up the small lymphatics. But the fact that the particles reaching the liver were few similarly in group B (Devastation of the diaphragm) and group C (Devastation of the diaphragm and the extirpation of the omentum), revealed little importance of absorption through the omentum.

As SIMER mentioned, the action of the macrophages which are present throughout the omentum seemed to form an efficient barrier against invasion of particulate matter.

Though TSUBOUCHI (1950) postulated that the maculas were also distributed in the thinner part of the membrane aloof from the blood vessels, we could not confirm the existence of the special structure, in which collagen fibers ramified reticulum fibrils and formed true net works like a sieve.

In regard to the mesenterium, only MATSUDA (1951) postulated that the particles passed through the cement substance between endothels of the membrane and were absorbed into venules or lymphatics, with coloured lymph nodes, at two to three hours after injection.

In our experiments, the absorption through the mesenterium could not always be recognized, but barely in a few instances. Greyish specks, locating along the blood vessels near the attachment of the small intestine, especially ileum, the sigmoid and the caecum, were consisted of accumulating carbon particles, part of which entered into perivascular fat tissue or ingulfed by macrophages. It seemed that a few particles were believed to enter into blood stream through the definite part of the mesenterium in some instances. However, neither lymphatics nor lymph nodes in the membrane were containing the particles.

It was revealed that the membrane was composed of wavy collagen fibers, weaving each other, and of fine down-like fibers. And the network of collagen was thick near the blood vessels where the argyrophilic fibers were found. But no particle was adherent to these fibers.

In other words, special structure for absorption i. e. macula cribriformis or milky spots could not be found anywhere on the mesenterium.

It was brought to light by our experiments that some particles penetrating the retroperitoneum could reach into the retroperitoneal cavity. The greyish specks which indicated the absorption sites were mostly found over the fat tissues near

the kidney.

Microscopic observation revealed that the particles were either accumulated in the subendothelial tissue with definite boundaries or arranged along the reticulum fibrils among fat cells. After entering into fat tissue, the particles are presumably absorbed by the two paths, venules and lymphatics. The venules distributing in the fat tissue were often surrounded by the particles. On the other hand, it could be a proof for lymphatic absorption that retroperitoneal lymph nodes, Lgll. renalis and Lgll. iliacae, were sometimes coloured with India ink, when Lgll. poplitae and Lgll.axillares were never containing the particles neglecting the supply by the blood stream.

The structural elements of the retroperitoneum resembled those of the mesenterium, quitely differing from those of the ventral peritoneum, and had not the macula cribriformis anywhere.

As for the serous surface of the digestive organs, MATSUDA (1951) postulated after phylogenetic investigation that absorption through the membrane into lymphatics and blood vessels took place in all kinds of vertebrates, except mammalia, and that the structure of these sites was meshes of collagen fibers, partly ramifying with reticulum fibrils.

From this point of view, the greyish colouring, locating in the narrowing rings of the caecum which was first demonstrated by our experimemts seemed to be a phylogenetic remain.

Presumably, the peculiar type of cells in the sites, seeming mesothelial, which does not formed capillaries, take definite role to fix the carbon particles. In other words, the particles in the subendothelial tissue are arranged within a tissue space walled by endothel-like cells incompletely. But the stites are not an effective pathway for the particles where the collagen fibers are neither woven each other, nor ramified with reticulum fibrils. When the inflammation has progressed intraperitoneally, the greyish colouring in the narrowing rings became broad and dense with accumulation of polymorphnuclears, monocytes and lymphocytes simultaneously.

SUMMARY

The carbon particles which are injected into peritoneal cavity are absorbed through the serous surface as follows:

1) The injected particles are removed exclusively by diaphragmatic lymphatics through conspicuously distributed macula cribriformis.

2) On the omentum, some particles are absorbed into venules and lymphatics chiefly distributing in the milky spots. We can not confirm the existence of macula cribriformis in the membrane.

3) A small dose of particle is absorbed at the narrowing rings of caecum, picturing dotted or complete circle around the bowel. It seemed to be a phylogenetic remain of absorption structure, which is active throughout serous surface of vertebrates besides mammalia.

4) Absorption of particles takes place, slightly in dose, in the retroperitoneum

over the fat tissue near the kidney.

5) In the mesenterium, it is believed that a few particles, forming greyish specks near the attachment of the intestine, enter into blood stream through the venule wall.

6) The macula cribriformis could not be detectable anywhere in the mesenterium, the parietal peritoneum, except diaphragm, and the serosa of digestive organs.

II. THE CHANGES OF LYMPHATIC ABSORPTION AFTER DESTRUCTION OF MAIN PATHWAY

As the foregoing paragraph had shown, particles injected into peritoneal cavity were exclusively absorbed through the macula cribriformis in the diaphragm.

This group of experiments was aimed to investigate the changes appearing on the absorption of particles after destruction of main pathway in the diaphragmatic aspect.

METHOD AND MATERIAL

1) Animals: Albino rabbits weighing approximately 1.5-2.0 kg.

2) Technical procedure: Two series of experiments were performed. The two groups of rabbits were designated B and C and operated under ether anesthesia as follows;

Group B: Devastation of peritoneal diaphragmatic surface by silver nitrate.

Group C: Extirpation of omentum and devastation of peritoneal diaphragmatic surface by silver nitrate.

The devasted diaphragm was adherent loosely to the liver surface after 24 hours and adhesion became more tightly, unable to peel the membrane, as the time lapsed. By this way, active diaphragmatic surfaces for absorption were closed almost completely.

Animals were injected with India ink intraperitoneally after various postoperative survivals and then bled to death at 0.5 to 4 hours.

Following lymph nodes were examined microscopically in all animals; Lgll. cardiacae, Lgll. art. pancreatico-lienalis, Lgll. art. hepaticae, Lgll. mesentericae craniales, Lgll. renales, Lgll. iliacae, Lgll. poplitae and Lgll. axillares. The lymph nodes of animals used in Part I were examined as control materials.

The stretched specimen of the parietal peritoneum and the mesenterium were fixed with neutral 10% formalin and stained by Bielschowsky-Maresch's method.

EXPERIMENTAL RESULT

The features appearing in the lymph nodes of control animals are shown in Table 1, where black coloured lymph nodes are shown as +++, greyish coloured lymph nodes as ++, lymph nodes containing a little carbon particles, proved only microscopically, as +, and lymph nodes free from carbon particle as − (Table 1, 2, 3).

Table 1 Carbon Particles Reaching Livers and Lymph Nodes of Sacrificed Rabbits in Group A. (Control Animals)

Rabbit No.	Interval sfrom Injection to Death	Liver	C. L.	P. L.	L. L.	M. L.	R. L.	I. L.	A. L.	Pp. L.
31	0.5 hr.	+	−	−	−	−	−	−	−	−
32	1 hr.	++	−	−	+	−	−	−	−	−
33	24 hrs.	+++	+	+	+	−	+	−	−	−
34	3 hrs.	+++	+	+	−	−	−	+	−	−
35	4 hrs.	+++	++	−	++	−	−	−	−	−
36	48 hrs.	+++	+	+	+	+	+	+	−	−
49	1 hr.	++	++	+	+	−	−	−	−	−
82	1/4 hr.	−	+	−	−	−	−	−	−	−
83	1 hr.	++	++	−	−	−	−	−	−	−
84	0.5 hr.	+	−	−	−	−	−	−	−	−
85	2.5 hrs.	+++	+++	++	++	−	−	−	−	−

Notes: C. L.; Lgll. cardiacae. P. L.; Lgll. art. pancreatico-lienalis.
L. L.; Lgll. art. hepaticae. M. L.; Lgll. mesentericae craniales.
R. L.; Lgll. renales. I. L.; Lgll. iliacae.
A. L.; Lgll. axillares. Pp. L.; Lgll. poplitae.

Table 2 Carbon Particles Reaching Livers and Lymph Nodes of Sacrificed Rabbits in Group B. (Devastation of Diaphragm by Silver Nitrate)

Rabbit No.	Intervals from Operation to Injection	Intervals from Injection to Death	Liver	C. L.	P. L.	L. L.	M. L.	R. L.	I. L.	A. L.	Pp. L.
39	3 ds.	4 hrs.	−	−	−	++	−	−	−	−	−
40	5 ds.	1 hr.	+	−	++	++	−	+	+	−	−
43	3 ds.	0.5 hr.	−	−	++	+	−	−	−	−	−
44	5 ds.	4.5 hrs.	−	++	++	++	−	++	−	−	−
52	24 ds.	1 hr.	−	−	+	−	−	−	+	−	−
57	7 ds.	0.5 hr.	+	+	+	+	+	+	−	−	−
62	31 ds.	3 hrs.	+	+	+	+	+	+++	+	−	−
74	14 ds.	1 hr.	−	−	+	−	−	−	++	−	−
76	3.5 hrs.	1 hr.	+	−	−	++	−	−	+	−	−
77	23 ds.	1 hr.	+	++	+	++	−	++	+	−	−

In brief, while the lymph nodes in the epigastric region, Lgll. cardiacae, Lgll. art. pancreatico-lienalis and Lgll. art. hepaticae, were containing carbon particles at the early stage, Lgll. mesentericae craniales, which are large lymph nodes mases filled with chyle, were not containing the particles with one exception, sacrificed in 48 hours after injection.

In the retroperitoneal cavity, Lgll. renales and Lgll. iliacae containing the particles were less than the lymph nodes in the epigastric region. Lgll. poplitae and Lgll. axillares never contained the particles.

In Group B and C, the features of epigastric lymph nodes were similar to

Table 3 Carbon Particles Reaching Livers and Lymph Nodes of Sacrificed Rabbits in Group C. (Devastation of Diaphragm and Extirpation of Omentum)

Rabbit No.	Intervals from Operation to Injection	Intervals from Injection to Death	Liver	C. L.	P. L.	L. L.	M. L.	R. L.	I. L.	A. L	Pp. L.
45	4 ds.	1 hr.	−	++	++	+	+	−	−	−	−
46	4 ds.	3 hrs.	−	+	+	+	+	−	−	−	−
47	7 ds.	3 hrs.	+	−	+	+	−	−	++	−	−
48	7 ds.	1 hr.	+	+	−	−	+	−	+	−	−
54	29 ds.	1 hr.	−	++	++	++	−	+++	+	−	−
60	30 ds.	3 hrs.	−	+++	++	−	++	−	−	−	−
65	26 ds.	1 hr.	−	−	++	−	++	+++	+	−	−
66	29 ds.	1 hr.	−	+	−	−	+	−	+	−	−
69	57 ds.	1 hr.	−	++	+++	++	+	+++	+++	−	−
73	12 ds.	1 hr.	−	+	+	+	++	+	−	−	−
78	25 hrs.	1 hr.	++	−	−	−	−	−	−	−	−
79	1 d.	1 hr.	+	−	−	−	−	++	−	−	−

those of control animals.

However, it seemed to be of considerable importance that the carbon particles were found in Lgll. mesentericae craniales more evidently and rapidly. The particles appeared in the medullary sinuses were not phagocytosed yet (Fig. 16).

The retroperitoneal lymph nodes were also coloured with India ink at the early stage after injection. Above all, the cases, having 1 or 2 months of survivals after operation and perfect closure of diaphragmatic aspect, had the deeply coloured lymph nodes and lymphatics which chained the retroperitoneal lymph nodes and entered into hiatus aorticus of the diaphragm (Fig. 17).

The fact that Lgll. poplitae and Lgll. axillares never contained the particles confirmed them reaching the lymph nodes, mentioned above, should be brought by lymphatics.

Ascites was found in varying degree, which was transparent and not stinky. Injected India ink appeared to be mingled with ascites homogenously, but in some cases formed clots or membranous substances over mesenteric folds.

The omentum of Group B, which often covered the operated wound or the liver lobe, were diffusely stained with the dye, having distinguished milky spots, in some cases which had been injected in 3 to 5 days after operation. Whereas, the membranes of cases which had the intervals of two weeks or more after operation were slightly coloured and microscopically had collagen fibers revealing the features of fibrinoid swelling.

In both Group B and C, it could be proved absorption through the peritoneum except diaphragm and omentum took place, though very slightly.

Greyish specks appeared in the definite parts of serous surface, that is, along blood vessels near the attachment of the intestine, over fat tissues near the kidney and the narrowing rings of the caecum.

Though the specks emerged more evidently and rapidly than in control animals, it seemed that the features were not induced by promoting absorption from these sites, but were induced by accumulating macrophages and increase of fibrin fixing the particles in situ (Fig. 18).

DISCUSSION

SAKAMOTO (1933) mentioned in his work concerning lymphatic system of rabbits that carbon particles were found in the epigastric lymph nodes at the early stage after injection, while they were slightly found in Lgll. mesentericae craniales in four days after injection.

This results were similar to those of our experiments.

It seemed to be one of compensative features resulting from diaphragmatic closure that Lgll. mesentericae craniales is containing free carbon particles in medullary sinuses in only half an hour after injection. This phenomenon is found in the cases of Group D and E, in which absorption of particles through the diaphragm is impaired significantly (Table 4, 5).

As Lgll. axillares and poplitea are never containing particles, the free ones appearing in the medullary sinuses may be brought by lymphatics.

However, lymphatics containing the particles could not be found throughout the mesenterium in these cases. Presumably the particles may reach the lymph nodes with retrograde flow after entering into lymphatics near the diaphragm.

Retroperitoneal lymphatic system of rabbits is poor. Lgll. renales and Lgll. iliacae were slightly coloured in one or two days after injection in control animals, and no lymphatics containing the particles was proved.

While these nodes subject to Group B and C are often coloured with the dye evidently and rapidly. The more perfect closure of diaphragmatic aspect continues, the more evident colouring of the nodes and lymphatics are found. We are of opinion that these features are analogous to those of Lgll. mesentericae craniales.

The particles may reach the retroperitoneal lymph nodes through the retroperitoneum. However, we could not confirm whether accumulating particles forming specks on the retroperitoneum are brought to these nodes.

Comparing with Group B and C, the latter included more cases with coloured superior mesenteric nodes than the former. It is not be able to maintain that the difference is induced by extirpation of omentum.

The fact that scarecely star like cell ingulfing the particles is detectable in both groups shows only a small amount of absorption through the omentum.

Besides diaphragm and omentum, greyish specks were often found in the definite parts of peritoneum, i. e. mesenterium near the attachment of the intestine, retroperitoneum near the kidney and narrowing rings of the caecum, more evidently and rapidly than in control animals.

Microscopic observation revealed that some of particles entered into subserous tissue but many particles were merely scattered over the membrane, probably fixed by fibrin, and ingulfed by macrophages.

Though the particles penetrating the retroperitoneum are expected to increase when the function of retroperitoneal lymph nodes is prosperous, it can not be determined by this series of experiments.

SUMMARY

1) When the diaphragmatic aspect has been blocked by operative procedure, Lgll. mesentericae craniales, Lgll. renales and Lgll. iliacae are often containing carbon particles more rapidly and evidently.

This seemed to be a compensative feature appearing in lymphatic absorption.

2) Small amount of particles are absorbed into subserous tissue at the definite parts of mesenterium, retroperitoneum and caecum as in control animals.

III. THE MICROSCOPIC FEATURES OF DIAPHRAGMATIC ASPECT IN THE INFLAMMED PERITONEAL CAVITIES

It is well known clinically that an inflammatory reaction occurring in the peritoneal cavity tends to localize in situ.

Opie (1929) reported experiments in which hemolytic streptococci, injected into the peritoneal cavities of rabbits, appeared within 10 minutes in the blood stream. If 24 hours prior to the injection of these bacteria, a sterile inflammatory irritant, such as aleuronat, had been injected into the peritoneal cavities, the organisms were prevent from reaching the blood stream.

While Menkin (1929) showed that if the inflammation, induced by aleuronat, had been in progress as long as 2 hours when blue trypan was injected, the appearance of blue trypan in the retrosternal lymph nodes was conspicuously less than in the lymph nodes of control animals.

Bangham (1953) suggested, thereafter, that lymphatic absorption was accelated at first, and then impeded in the acute peritonitis.

These literatures mentioned that the phenomenon was induced by humoral and cellular mechanism in the inflammed peritoneal cavity, but did not described morphologic change of main absorption path, i. e. diaphragm.

From this point of view, I tend to investigate the change of macula cribriformis appearing in the diaphragm with peritonitis.

METHOD AND MATERIAL

1) Animals. Albino rabbits, weighing approximately 1.5-2.0 kg.

2) Technical procedures. The two series of experiments were performed.

The two groups of rabbits used were designated D and E.

The animals subject to Group D were injected with terpentine oil, 0.1 g per kg of bodyweight diluted by physiological saline intraperitoneally.

In Group E, extirpation of omentum were performed under ether anesthesia. After these procedures, India ink was injected into peritoneal cavities at various intervals. Animals survived for 0.5 to 5 hours prior to death.

The stretched specimens of diaphragm, omentum and other parts of peritoneum

were fixed in 10% formalin and stained by Bielschowsky-Maresch's method.

The retrosternal lymph nodes and the liver were used to compare the amounts of carbon particles passing through the diaphragm.

Table 4 Carbon Particles Reaching Livers and Lymph Nodes of Sacrificed Rabbits in Group D. (Sterile Peritontiis Induced by Terpentine Oil)

Rabbit No.	Intervals from Injection of Irritant to next Inj.	Intervals from Injection of India Ink to Death	Liver	C. L.	P. L.	L. L.	M. L.	R. L.	I. L.	A. L.	Pp. L.
55	28 hrs.	1 hr.	−	−	−	−	−	−	−	−	−
56	2 ds.	1 hr.	−	−	−	−	−	−	−	−	−
59	2 ds.	3 hrs.	+	++	−	++	++	++	−	−	−
63	4 hrs.	1 hr.	−	−	−	−	−	−	−	−	−
67	1 hr.	1 hr.	++	++	++	−	−	+	−	−	−
68	4 ds.	1.5 hr.	−	++	+	−	+	−	+	−	−
70	7 ds.	1 hr.	−	−	−	+	−	−	−	−	−
80	14 ds.	1 hr.	+	−	+	−	+	++	++	−	−
81	10 hrs.	1 hr.	−	−	−	−	+	−	−	−	−

EXPERIMENTAL RESULT

Group D (Table 4) : Acute inflammatory change, such as plentiful ascites, transparent and with terpentine odour, and turbidity of peritoneum were seen in varying degree. Necrotic spots induced by terpentine oil occasionally with hemorrhagic change, were detectable on the retroperitoneum, the omentum and the serosa of digestive organs in some cases.

Clumps or precipitates of India ink were conspicuous on the peritoneal aspect of the diaphragm, having no adhesion with the liver surface.

Comparing with the control animals, the radial black stripes among tendon bundles became obscure and the subserous lymphatics containing India ink were more sparsely.

In the case of No. 67, in which the inflammation of peritoneal cavity had progressed for two hours, the macula cribriformis kept fine structure of reticulum fibrils and was covered partly by fibrin meshes with numerous mononuclears. Of this case, the retrosternal lymph nodes, the liver and the spleen were fully containing carbon particles (Fig. 20).

However, in the case of No. 63, in which the inflammation of peritoneal cavity had progressed for five hours, the carbon particles reaching the retrosternal lymph nodes and the liver were strikingly less than in No. 67, notwithstanding normal feature of macula cribriformis.

When ten hours or more passed after injection of irritant, the collagen fibers of the diaphragm were severely altered in microscopic observation. The collagen change of the nature was so-called fibrinoid swelling. And fine reticulum fibrils, structuralel ements of the macula cribriformis, were replaced by thick fibers and

they loosed argyrophilic nature. As a result of these changes, the characteristic features revealed were concentric luminal narrowing of macula cribriformis and no inflammatory cellular remnants. Few particles were accumulating over the membrane (Fig. 23, 24, 25, 26, 27). This significant feature of macula cribriformis was found even in two weeks after injection of irritant.

In these cases, the retrosternal lymph nodes either failed to show the presence of India ink, or occasionally showed it only traces.

In control animals, the livers were dyed with India ink purplish brown to greyish brown in accordance with the lapse of the time.

Namely, at 30 minutes after injection, the liver had yet physiologic colour, at 1 hour, it was greyish, and, at 3 hours, it was dark greyish.

Microscopically, injected carbon particles were not seen at 15 minutes, however, at 30 minutes or more, Kupffer's cells, fully ingulfing carbon particles, were seen in the lobules of the liver, whereas, sinusoid cells were containg a little carbon particles (Fig. 19).

The particles reaching the liver seemed to be proportional to them passing through the diaphragm.

However, the livers of this group were containing few carbon particles in the lobules, except a case in the early stage of inflammtion (Fig. 21).

It could not be determined in this series that absorption of the particles through the omentum is increased or decreased.

But the collagen fibers of the membrane which was in the inflammed peritoneal cavity for sevral hours or more became thick and the particles accumulating on the milky spots were less than in control animals.

Table 5 Carbon Particles Reaching Livers and Lymph Nodes of Sacrificed Rabbits in Group E. (Extirpation of Omentum)

Rabbit No.	Intervals from Operation to Injection	Intervals from Injection to Death	Liver	C. L.	P. L.	L. L.	M. L.	R. L.	I. L.	A. L.	Pp. L.
37	3 ds.	1 hr.	+	−	−	++	++	+	−	−	−
38	5 ds.	3 hr.	++	++	−	++	−	−	−	−	−
41	5 ds.	0.5 hr.	−	+	++	++	++	++	++	−	−
42	3 ds.	5 hrs.	+	+	+	−	−	++	++	−	−
53	26 ds.	1 hr.	+	++	+	−	−	+	++	−	−
71	7 ds.	1 hr.	+	−	−	+	−	−	−	−	−
72	1 d.	1 hr.	−	−	−	−	−	−	−	−	−
75	3 hrs.	1 hr.	−	−	−	−	−	++	−	−	−

Group E (Table 5): Ascites was detectable in varying degree, which was transparent and not stinky. Injected India ink mingled with ascites homogenously, but in a few cases, partly formed clots in recess or membranous substances over serous surface.

Diaphragmatic surface was not adherent to the liver and was coloured with

radial black stripes among tendon bundles and subendothelial lymphatics containing the particles. By the silver impregnation method, macula cribriformis mostly kept fine net work of reticulum fibrils.

But in a few cases, they showed concentric luminal narrowing due to fibrinoid swelling of fibrous elements and diminution of argyrophilic nature of reticulum fibrils. Sometimes it could be seen that fibrin meshes with numerous mononuclears blocked the macula cribriformis (Fig. 28, 29). The retrosternal lymph nodes were fairly conspicuous staining with India ink. However, the amounts of particles reaching the livers were clearly less than in control animals. In other words, at 1 hour after injection, merely a few star like cells ingulfing the particles were found in the lobules of cases, which had survived for 3 hours to 7 days after operation (Fig. 22).

The omental absorption of particles was also taken place through the venules, but the amount of them reaching the liver was only a little, as foregoing paragraph had shown.

By this reason, it seemed that the sparse distribution of stained Kupffer's cells in the liver lobule was induced by the decrease of particles passing through the diaphragm. Namely, the removal of the particles was impeded by an inflammatory reaction postoperatively.

This view was supported by the fact that many Kupffer's cells fully ingulfing the particles were seen in one case, which had 26 days prior to death and failed postoperative peritonitis already, at 1 hour after injection of the dye as well as control animals.

DISCUSSION

By measuring the clearance of particles, amorphous and spherical radioactive glass particles containing caesium,[174] from peritoneal cavities in which inflammation of varying intensity and maturity had been induced, Bangham (1953) showed in the rats, 1 hour after the injection of a small dose terpentine a peritonitis was present, but no reduction in clearance of particles was found. After the same dose had been in contact with the peritoneum for 24 hours, however, there was a significant fall.

In severe hemorrhagic peritonitis produced by massive injection of terpentine, there was almost no movement of particles from peritoneal cavity to retrosternal lymph nodes.

In our experiments, the dose of terpentine oil injected intraperitoneally was far less than that in Bangham's work.

The carbon particles, which were injected into inflammatory peritoneal cavity at 1 hour after injection of irritant, soon appeared conspicuously in the retrosternal lymph nodes, liver and spleen as control animals.

While the particles which were injected in 5 hours after injection of irritant, were checked in reaching these organs. And this inhibition was proved in one case which had terpentine peritonitis for two weeks.

The decrease of the rate, though slightly, at which particles leave the peritoneal cavities of Group E seemed to be induced by an inflammation after operative procedure.

Concerning the reason why the absorption of particulate matter from peritoneal cavity was impeded in the inflammatory process, Zinser and PRYDE (1952) commented that deposition of fibrin on the peritoneal surface impaired access to the lymphatics, and Menkin also suggested the lymphatics were blocked by fibrin clots.

In our experiments, clumps or precipitates of India ink were conspicuous over serous surface and fine fibrin meshes with many macrophages were adherent to macula cribriformis. The inhibition proved in postoperative peritonitis or in the early stage of terpentine peritonitis seemed to be induced by increase of fibrin in the peritoneal cavity.

However, we are of opinion, in addition to these factors, that concentric luminal narrowing of macula cribriformis, which is induced by fibrinoid swelling of collagen fibers in the peritoneal diaphragmatic aspect,may be an effective barrier for the removal of particles through the pathway. This marked features of macula cribriformis impair the absorption of the particles not only by diminution of effective area but also by changed nature of reticulum fibrils, which seemed to be of important role for absorption.

Though YAMAMOTO (1956) mentioned these microscopic changes of macula cribriformis could be induced as an allergic response to protein fraction of the destructed tubercle bacilli or horse serum, they should be generally considered as a state of defensive reaction resulting from an intraperitoneal inflammation.

SUMMARY

The removal of particulate matter from inflammed peritoneal cavities, induced by a small dose of terpentine oil, is accelerated in the early stages, and is strikingly impaired after several hours or more.

We tend to conclude that this defensive response, to some extent, depends on the marked luminal narrowing of macula cribriformis appearing in the diaphragm.

The local fixation of particles may be performed by increase of fibrin over serous surface in postoperative process and in the early stage of terpentine peritonitis, where morphological changes of macula cribriformis are slight or scarce.

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和 文 抄 録

腹膜腔の異物小粒子吸収に関する実験的研究

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井 谷 幹 一

I) 哺乳動物の腹膜腔に注入された異物小粒子が横隔膜腹膜面を通過してリンパ管内に吸収されることは古くから知られていた．木原教授はこの部分に膠原線維を枠とし，網状線維を網目とする特殊な線維構造を見出し，これが漿膜内皮からリンパ毛細管までの脈管外通液路を形成し，異物小粒子の移動路となつていることをみとめ，篩状斑と命名した．

私は家兎腹腔墨汁注入法によつて墨汁粒子が主として，横隔膜篩状斑を通じて吸収されることを再確認するとともに横隔膜以外の腹膜の吸収状況および線維構造を精査し，次の部分からも僅かながら墨粒子が漿膜下組織に移行し盲腸漿膜以外では小静脈或はリンパ毛細管に吸収されることを確めた．すなわち ①大網，とくに乳斑部．②腸間膜，腸管附着部附近の血管係蹄周囲．③後壁腹膜，腎周囲脂肪組織を蔽う部分．④盲腸絞軋輪部．には種々の程度に墨斑がみられるが，大網以外は毎常出現するとは限らず，墨染の度合も微弱である．大網，腸間膜．後壁腹膜は交織した膠原線維とその間に介在する羽毛状の好銀性の乏しい細線維から成つているが篩状斑のごとく真の網目を形成しているところはない．

盲腸絞軋輪部の墨斑は鳥類以下の脊椎動物に一般的にみられる胃腸管漿膜の異物吸収能の発生学的な名残りと考えられるが，特殊な網状構造はみとめ得ない．

II) 横隔膜腹膜を硝酸銀で腐蝕し肝表面との間に癒着を生ぜしめ，通液路を遮断した家兎では腹腔内リンパ節，とくに腸間膜根リンパ節，後腹膜腔の腎動脈リンパ節，腸骨リンパ節などの機能が代償的に亢進し，注入墨粒子によつて早期に且，著明に着色される．

III) 少量のテルベンチン油を注入して腹膜炎を惹起せしめた家兎では，墨粒子の横隔膜からリンパ路への移行が，数時間後には著明に減少する．起炎物質注入後10時間以上経過すると横隔膜篩状斑は線維成分の膨化によつて小孔状と化し，吸収面積が著るしく狭くなつているのを観ることができる．このことは炎症に伴う，自衛反応の一種と解することができよう．

テルベンチン油腹膜炎で篩状斑の変化が未だ現われない期間，あるいは手術後（大網切除）にも横隔膜吸収の減少をみるが，これは Pryde Menkin なども指摘したように主として漿膜面の線維素増加によるものと考えられる．

Fig. 1 Normal macula cribriformis Pars tendinea diaphragmatica ×200

Fig. 2 Normal macula cribriformis near Vv. diaphragmatica ×200

Fig. 3 Omentum, composed of collagen fibers and fine fibrils, but no formation of macula ×200

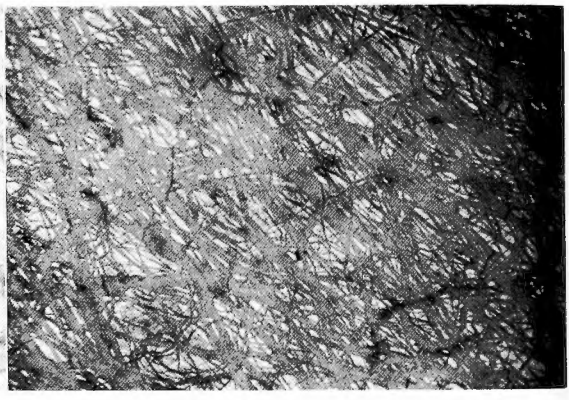

Fig. 4 Mesenterium of small intestine ×200

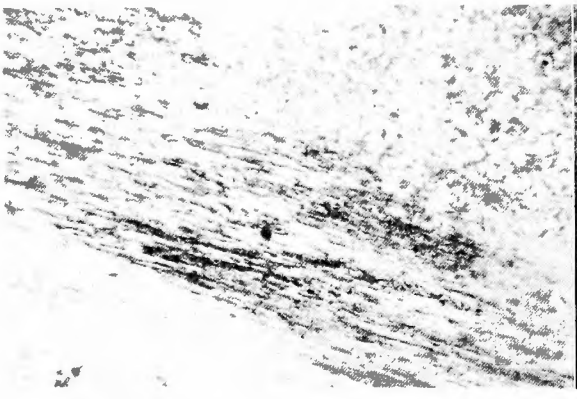

Fig. 5 Mesenterium of small intestine, carbon particles accumulating in subserous tissue ×200

Fig. 6 Mesenterium of caectm ×200

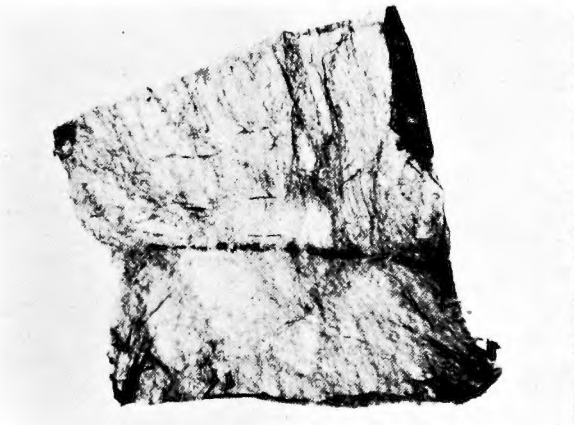

Fig. 7 Linear colouring appearing over narrowing ring of caecum

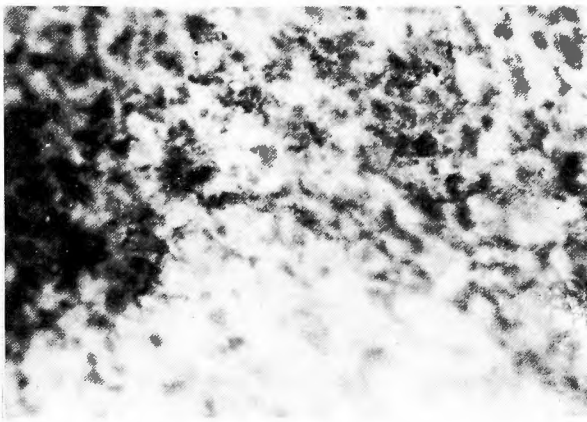

Fig. 8 Carbon particles entering into subserous tissue of narrowing ring of caecum ×400

Fig. 9 Fibrous structure of caecum serosa ×200

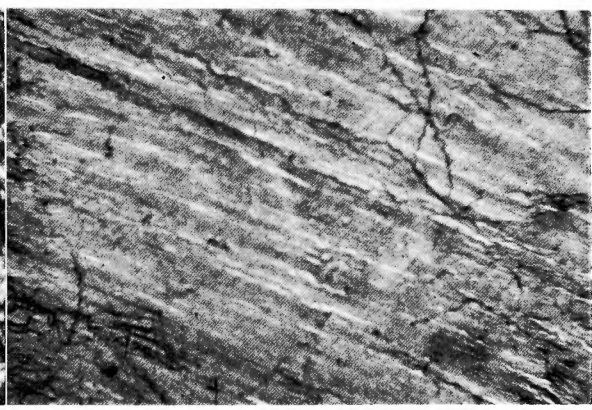

Fig. 10 Ventral parietal peritoneum ×200

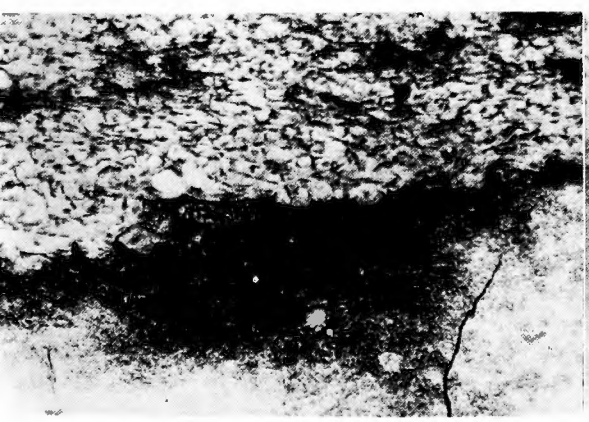

Fig. 11 Subendothelial tissue of retroperitoneum, carbon particles accumulating conspicuously ×200

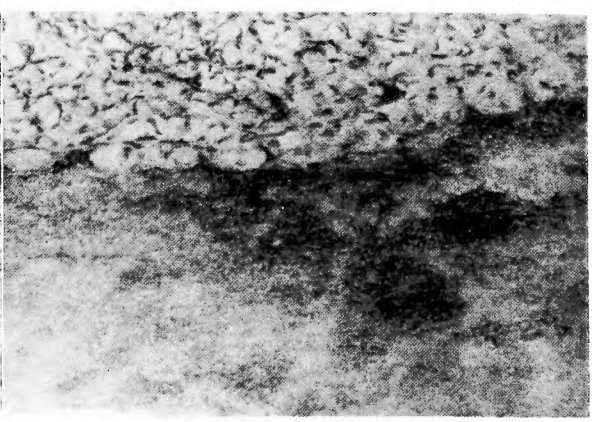

Fig. 12 Subendothelial tissue of retroperitoneum ×200

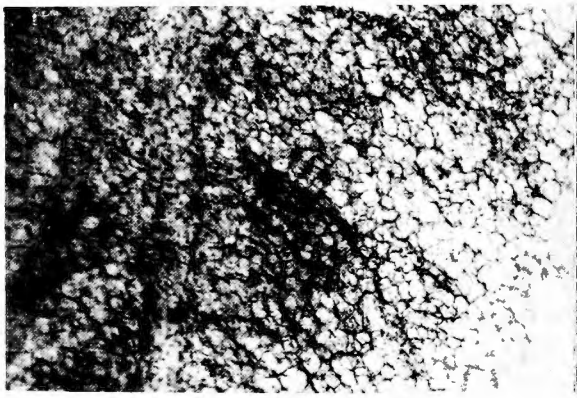

Fig. 13 Subendothelial tissue of retroperitoneum, fat cells being outlined by particles ×200

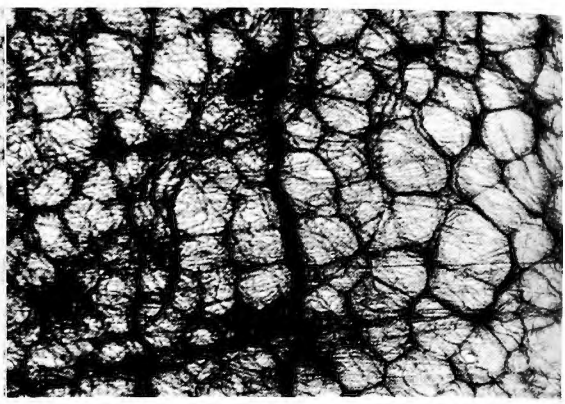

Fig. 14 Carbon particles, adherent to reticulum fibers among fat cells ×400

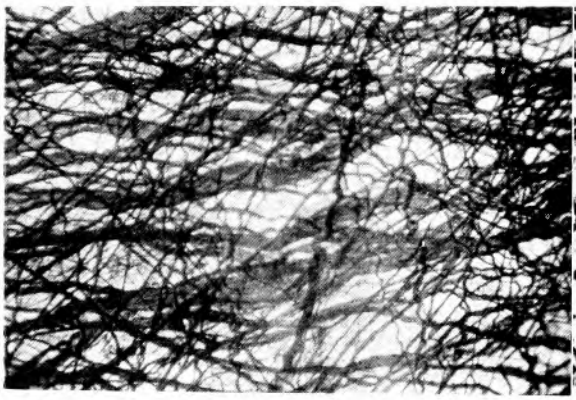

Fig. 15 Fibrous elements of retroperitoneum ×200

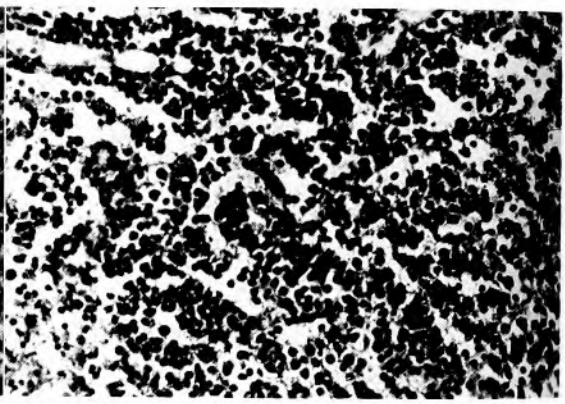

Fig. 16 Free carbon particles accumulating in medullary sinus of Lgll. mesentericae craniales of Group C ×200

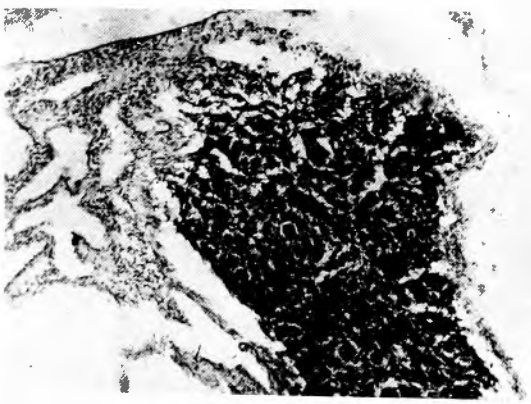

Fig. 17 Deeply coloured renal lymph node of Group C ×100

Fig. 18 markedly stained mesenterium of small intestine of Group C

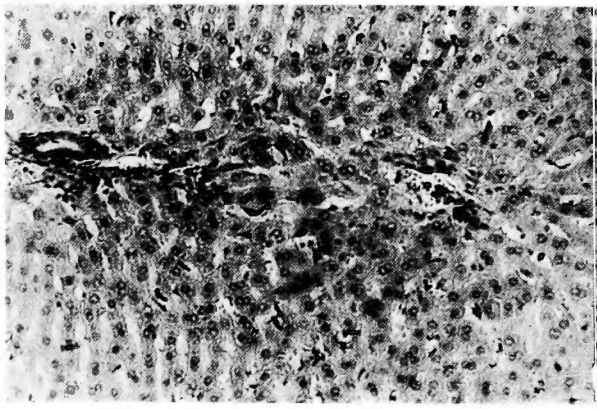

Fig. 19 Normal liver, 1 hour after injection of India ink, appearing many star like cells ingulfing carbon particles ×200

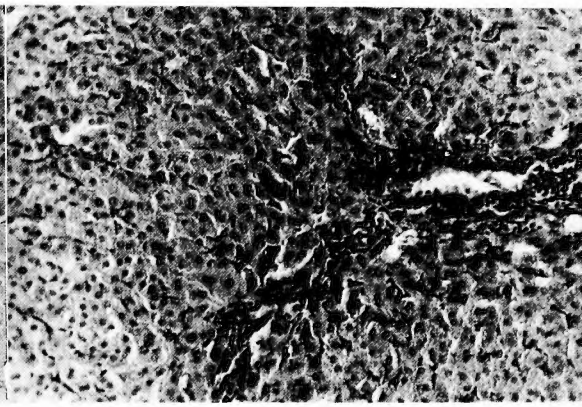

Fig. 20 Liver of Group D, india ink being injected I hour after injection of irritant ×200

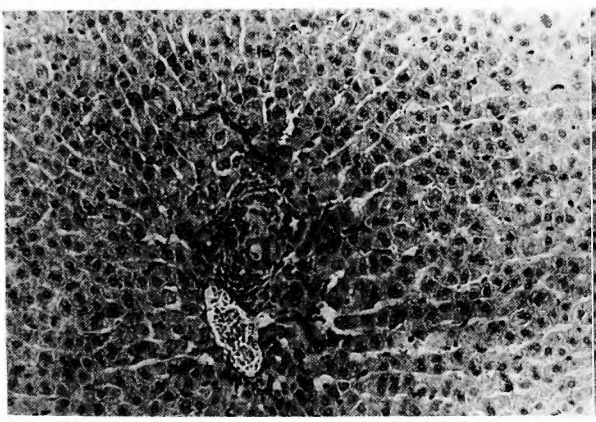

Fig. 21 Liver of Group D, India ink being injected 10 hours after injection of irritant ×200

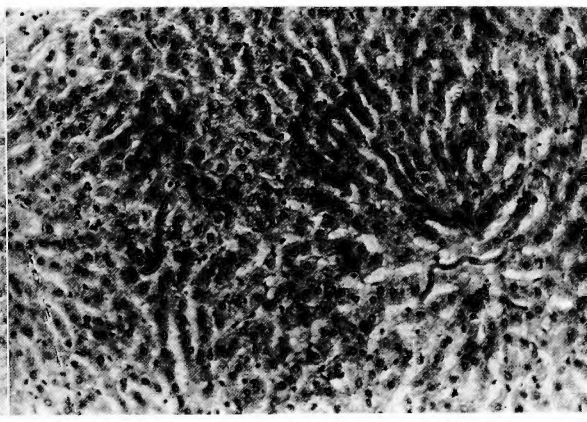

Fig. 22 Liver of Group E, India ink being injected 1 day after extirpation of omentum ×200

Fig 23 Marked luminal narrowing of macula cribriformis, Group D

Fig. 24 Marked luminal narrowing of macula cribrriformis, Group D

Fig. 25 Marked luminal narrowing of macula cribriformis, Group D ×200

Fig. 26 Marked luminal narrowing of macula cribriformis, Group D ×200

Fig. 27 Luminal narrowing of macula cribriformis, Group D ×200

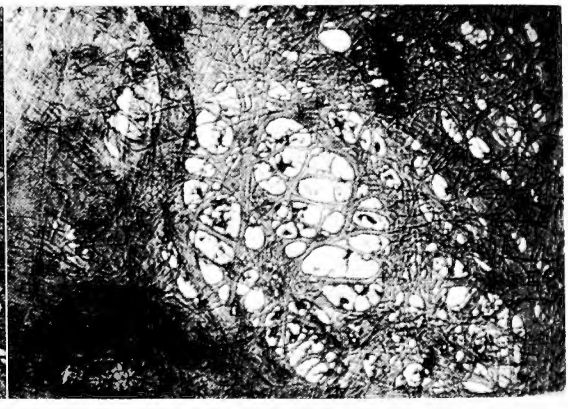

Fig. 28 Slight luminal narrowing and diminution of reticulum fibrils of macula cribriformis, Group E ×200

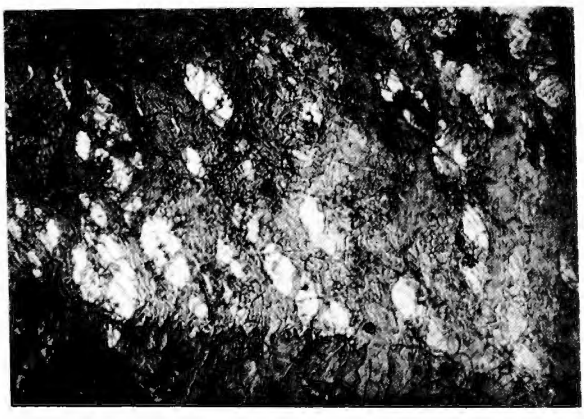

Fig. 29 Slight luminal narrowing of macula cribriformis, Group E ×200